that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α-fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α-fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host- related factors should facilitate the development of more effective therapeutic regimens.

Acknowledgement

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

References

1 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.

2 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.

3 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–1695.

4 Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE: Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol 2007;81:8211–8224.

5 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 2007;46:1357–1364.

6 Fishman SL, Factor SH, Balestrieri C, Fan X, Dibisceglie AM, Desai SM, Benson G, Branch AD: Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. Clin Cancer Res 2009;15:3205–3213.

7 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C: Comparison of full-length sequences of interferon sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.

8 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.

9 El-Shamy A, Sasayama M, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Prediction of efficient virological response to pegylated interferon/ribavirin combination therapy by NS5A sequences of hepatitis C virus and anti-NS5A antibodies in pre-treatment sera. Microbiol Immunol 2007;51:471–482.

10 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 2008;48:38–47.
11 Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E: Nagano Interferon Treatment Research Group: Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 2008;48:1753–1760.
12 Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, Aikata H, Takahashi S, Chayama K: Hiroshima Liver Study Group: Randomized trial of high-dose interferon-α-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol 2009;81:640–649.
13 Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB: Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
14 Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M: Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
15 Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J: *IL28B* is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nat Genet 2009;41:1100–1104.
16 Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY: Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study: Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138:1338–1345.
17 Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M: Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.
18 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H: Amino acid substitution in HCV core region and genetic variation near the interleukin-28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 2010;52:421–429.
19 Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K: Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 1990;87:9524–9528.
20 Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y: A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 2001;46:471–477.
21 Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakitani S, Teshima R, Nishioka Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K: Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395–402.
22 Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, Sugiyama M, Matsuura K, Sugauchi F, Asahina Y, Nakagawa M, Watanabe M, Sakamoto M, Maekawa S, Sakai A, Kaneko S, Ito K, Masaki N, Tokunaga K, Izumi N, Mizokami M: Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol 2011;54:439–448.
23 Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K: HCV substitutions and *IL28B* polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 2011;60:261–267.
24 McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ: PROVE1 Study Team: Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827–1838.
25 McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM: PROVE3 Study Team: Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010;362:1292–1303.
26 Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S: PROVE2 Study Team: Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.
27 Jouet P, Roudot-Thoraval F, Dhumeaux D, Metreau JM: Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis. Gastroenterology 1994;106:686–690.
28 Poynard T, McHutchinson J, Goodman Z, Ling MH, Albrecht J: Is an 'a la carte' combination interferon alfa-2b plus ribavirin regimen possible for the first-line treatment in patients with chronic hepatitis C? The ALGOVIRC Group. Hepatology 2000;31:211–218.
29 Bruno S, Camma C, Di Marco V, Rumi M, Vinci M, Camozzi M, Rebucci C, Di Bona D, Colombo M, Craxi A, Mondelli MU, Pinzello G: Peginterferon alfa-2b plus ribavirin for naïve patients with genotype 1 chronic hepatitis C: a randomized controlled trial. J Hepatol 2004;41:474–481.
30 Bayati N, Silverman AL, Gordon SC: Serum α-fetoprotein levels and liver histology in patients with chronic hepatitis C. Am J Gastroenterol 1998;93:2452–2456.
31 Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD: Clinical, virological, and pathologic significance of elevated serum α-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol 2001;32:240–244.
32 Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T: Clinical significance of elevated α-fetoprotein in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 2004;99:860–865.
33 McHutchison JG, Poynard T, Pianko S, Gordon SC, Reid AE, Dienstag J, Morgan T, Yao R, Albrecht J: The impact of interferon plus ribavirin on response to therapy in black patients with chronic hepatitis C. The International Hepatitis Interventional Therapy Group. Gastroenterology 2000;119:1317–1323.
34 Kaplan DE, Sugimoto K, Ikeda F, Stadanlick J, Valiga M, Shetty K, Reddy KR, Chang KM: T-cell response relative to genotype and ethnicity during antiviral therapy for chronic hepatitis C. Hepatology 2005;41:1365–1375.
35 Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T: Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 1999;30:1014–1022.

HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy

C Nelson Hayes,[1,2] Mariko Kobayashi,[3] Norio Akuta,[3] Fumitaka Suzuki,[3] Hiromitsu Kumada,[3] Hiromi Abe,[1,2] Daiki Miki,[1,2] Michio Imamura,[1,2] Hidenori Ochi,[1,2] Naoyuki Kamatani,[4] Yusuke Nakamura,[5] Kazuaki Chayama[1,2]

[1]Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima, Japan
[2]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
[3]Department of Hepatology, Toranomon Hospital, Tokyo, Japan
[4]Center for Genomic Medicine, Riken, Yokohama, Japan
[5]Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan

Correspondence to
Professor Kazuaki Chayama, Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan; chayama@hiroshima-u.ac.jp

Revised 21 September 2010
Accepted 26 September 2010
Published Online First 10 November 2010

ABSTRACT

Background and aims A number of recent studies have shown that human polymorphisms near the *IL28B* type III interferon (IFNλ) gene influence the response to peg-interferon plus ribavirin combination therapy for infection with chronic hepatitis C virus (HCV). Viral polymorphisms, including substitutions within the HCV core and NS5A proteins, have also been shown to influence treatment outcome, but it is not known whether these factors act independently of the IL28B polymorphism or if they reflect the same or a different underlying mechanism. Multiple logistic regression was used to determine whether host and viral polymorphisms independently predict sustained virological response (SVR).

Methods Two single nucleotide polymorphisms were genotyped in the IL28B locus (rs12979860 and rs8099917) from 817 patients with chronic HCV infection, and substitutions at amino acids 70 and 91 of the HCV core protein and within the NS5A interferon sensitivity-determining region (ISDR) were analysed.

Results It was found that independent predictors of an SVR included IL28B rs12979860 CC genotype (OR=4.98; p=4.00E-08), core amino acid 70 substitutions (OR=0.53; p=0.016), age and baseline viral load. For non-virological response, the IL28B rs12979860 CT/TT genotype (OR=0.23; p=1.96E-8) and age were independent predictors. IL28B rs12979860 genotype (p=1.4E-8), core amino acid 70 substitutions (p=0.0013), ISDR substitutions (p=0.0019), baseline viral load, γ-glutamyltranspeptidase, alanine aminotransferase and platelet count were independent predictors for change in viral load by week 4 of treatment.

Conclusions IL28B polymorphisms and HCV core amino acid 70 substitutions contribute independently to an SVR to peg-interferon plus ribavirin combination therapy.

Significance of this study

What is already known about this subject?
- Clinical and viral factors influence the outcome of peg-interferon plus ribavirin combination therapy for chronic hepatitis C virus infection.
- Polymorphisms within the human IL28B locus strongly influence treatment outcome.
- Substitutions at amino acids 70 and 91 of the HCV core protein as well as within the interferon sensitivity-determining region (ISDR) also affect response to treatment.

What are the new findings?
- IL28B polymorphisms as well as substitutions at amino acid 70 both independently predict sustained virological response, suggesting that they influence treatment outcome through different mechanisms.
- IL28B polymorphisms, substitutions at core protein amino acid 70 and ISDR substitutions are each independent predictors for change in viral load after 4 weeks of treatment.

How might it impact on clinical practice in the forseeable future?
- The combination of IL28B genotyping and detection of core protein substitutions may yield more accurate pretreatment predictions of treatment efficacy.

INTRODUCTION

Hepatitis C virus (HCV) is a primary cause of chronic hepatitis and often progresses to liver cirrhosis and hepatocellular carcinoma.[1 2] Peg-interferon plus ribavirin combination therapy (PEG-RBV) is the current standard of care, but it is only effective in 50% of patients and has severe side effects often requiring discontinuation or dose modification.[3] Consequently, reliable predictors are needed to identify unsuitable candidates as early as possible.

Genome-wide association studies have reported common single nucleotide polymorphisms (SNPs) predictive of response to interferon treatment. While polymorphisms in MxA,[4 5] interferon α-receptor 1,[6] osteopontin[7] and MAPKAPK3[8] have been reported to be associated with interferon response, several linked SNPs within the IL28B locus on chromosome 19 have recently been shown to be the strongest predictors of early viral kinetics, response to treatment and spontaneous viral clearance.[9–15]

Viral polymorphisms have also been shown to be associated with treatment response. HCV genotypes 1 and 4 in particular are considered more difficult to treat than genotypes 2 and 3,[16 17] and genotype 3 is associated with steatosis.[18] Within genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity-determining region (ISDR) of the NS5A protein[19 20] have also been shown to be associated with treatment outcome, especially among Japanese patients.

Consequently, a number of human and viral factors are now known to affect response to treatment, but in order to identify the most important independent predictors and to identify which, if any, may be useful in guiding clinical practice, it is necessary to analyse them simultaneously in a multivariate model. In this study we therefore attempted to identify host and viral factors that independently predict treatment outcome.

MATERIALS AND METHODS

Patients

Data from 817 patients who were treated with PEG-RBV combination therapy for chronic hepatitis C genotype 1b infection between 2002 and 2008 were collected from Toranomon Hospital (Tokyo) and hospitals that belong to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Study subjects tested positive for HCV RNA over a span of >6 months, were negative for hepatitis B and HIV, and showed no evidence of other liver diseases. Patients received weekly injections of peg-interferon-α2b at 1.5 g/kg body weight for 48 weeks and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients with low baseline viral load (<5 log IU/ml) were excluded, as were patients who received <0.89 g/kg of peg-interferon or <8.3 mg/kg of ribavirin. Treatment success was evaluated based on a sustained virological response (SVR), defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (TR or relapser), in which HCV RNA dropped to undetectable levels during treatment but then later rebounded. In those with a non-viral response (NVR), HCV RNA levels failed to decline by 2 log10 IU/ml by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made according to the criteria of Desmet *et al.*[21] All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA levels

HCV RNA levels were monitored throughout the course of treatment at 1 or 2 month intervals for a total of at least six time points via reverse transcription–PCR (RT–PCR) using the original Amplicor method, the high range method or the TaqMan RT–PCR test. The measurement ranges of these assays were 0.5–850 kIU/ml, 5–5000 kIU/ml and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline (PBS) and reanalysed. All values were reported as log IU/ml.

ISDR and core amino acid substitutions

Amino acid substitutions in the HCV core and ISDRs were determined by direct sequencing of PCR products following extraction and reverse transcription of serum HCV RNA. Core amino acid substitutions at positions 70 and 91 (core70 and core91) were determined according to Akuta *et al*,[22 23] and the number of ISDR substitutions was established as in Enomoto *et al.*[19 21 24] Of the 817 patients in the study, substitutions for both ISDR and core70 could be determined for 379 patients.

SNP genotyping

We genotyped each patient for two IL28B SNPs previously reported to be associated with treatment outcome, rs12979860 and rs8099917.[9–11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously.[25 26] The two SNPs are in strong linkage disequilibrium, with a correlation coefficient of 0.99. SNP genotypes for both rs12979860 and rs8099917 were determined for 815 patients (99.7%).

Statistical analysis

All analyses were performed using the R statistical package (http://www.r-project.org). Non-parametric tests (χ^2 and Mann–Whitney U tests) were used to detect significant associations. All statistical analyses were two sided, and $p<0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors using $p<0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on Akaike Information Criterion (AIC) score and validated using the rms package in R. ORs and 95% CIs were calculated for each factor.

RESULTS

Patient characteristics

Patient profiles are shown in table 1. Forty-five per cent of patients achieved an SVR, 22% were transient responders and 33% failed to respond to treatment (NVR). Males were significantly more likely to achieve an SVR than females (50% and 38%, respectively; p=0.0011), and younger patients were more likely to achieve an SVR than older patients (59.2% and 40.9% above and below median age 58, respectively; p=1.57E-6). Patients who achieved an SVR also had lower γ-glutamyl-transpeptidase (γGTP) levels (36 IU/l vs 45 IU/l; p= 0.008) and higher platelet counts (17.1 vs 15.3×10^{10}/L; p=3.649E-05) than those who did not.

IL28B SNP genotypes

The genotypes of two IL28B SNPs were measured for each patient. Because of linkage disequilibrium, SNP results are nearly interchangeable. However, six patients showed an intermediate haplotype consisting of the favourable genotype for rs8099917 (TT) but an unfavourable genotype for rs12979860 (CT), whereas only one of the six patients achieved an SVR, suggesting that rs12979860 is a better predictor of SVR in this data set.

The frequency of the risk allele (T) for rs12979860 was 0.15 among all patients and 0.08 in SVR patients, 0.14 in TR patients and 0.27 in NVR patients. Patients homozygous for the rs12979860 favourable allele (CC) were significantly more likely to achieve an SVR compared with those with TC or TT genotypes (53% vs 24%, OR=3.55, p=3.95E-13). Conversely, patients with the risk allele (TC or TT) were significantly more likely to show an NVR (55% vs 25%; OR=0.265; p=4.4E-16). Patients with the rs12979860 CC genotype had a marginally lower baseline viral load (6.6 vs 6.4 log IU/ml; p=0.093), but showed significantly greater reduction in viral load by week 4 of treatment (−3.2 vs −0.8 log IU/ml; p<2.2E-16). The rs12979860 CC genotype was also associated with wild type core70 (78% vs 54%; p=1.6E-6) and non-wild type ISDR (67% vs 83%; p=0.007).

The frequency of the rs8099917 risk allele (G) was 0.15 among all patients, 0.08 in SVR patients, 0.13 in TR patients and 0.26 in NVR patients. Patients with the rs8099917 TT genotype were significantly more likely to achieve an SVR than patients with GT or GG genotypes (53% vs 24%, OR=3.43, p=2.18E-12), and GT/GG patients were significantly more likely to show an NVR

Table 1 Patient profiles by response to treatment

	All (813)	SVR (366)	TR (176)	NVR (271)
Sex (M/F)	459/354	231/135	84/92	144/127
Age	58 (51–65)	56 (47–63)	60.5 (56–65.25)	59 (52.5–66)
Body weight (kg)	59 (52–67)	60 (52–68.25)	58 (51–66)	60 (52–66.4)
BMI (kg/m^2)	22.61 (20.81–24.65)	22.44 (20.46–24.58)	22.85 (20.85–24.89)	22.76 (21.12–24.63)
Hypertension (yes/no)	141/672	61/305	29/147	51/220
Diabetes (yes/no)	97/716	31/335	25/151	41/230
Fibrosis (0–2/3–4)	138/421	52/227	34/81	52/113
Activity (0–1/2–3)	274/272	136/138	53/56	85/78
ISDR (0, 1/≥2)	78/298	43/128	15/71	20/99
Amino acid 70 (wild-type/mutant)	256/139	137/45	54/35	65/59
Amino acid 91 (wild-type/mutant)	221/178	112/72	51/40	58/66
WBC (/L)	4.71×10^9 (3.9×10^9–5.7×10^9)	4.9×10^9 (4.0×10^9–6.0×10^9)	4.6×10^9 (3.8×10^9–5.4×10^9)	4.6×10^9 (3.7×10^9–5.5×10^9)
Haemoglobin (g/dl)	14.1 (13.2–15)	14.2 (13.3–15.22)	13.9 (13.1–14.8)	14.1 (13.05–14.9)
Platelets ($\times10^4$L)	16.1×10^6 (12.5×10^6–19.9×10^6)	17.1×10^6 (13.7×10^6–20.7×10^6)	15.5×10^6 (11.3×10^6–18.8×10^6)	15.1×10^6 (12×10^6–19.2×10^6)
AST (IU/l)	45 (34–65.5)	43 (32.25–64)	43.5 (33.25–66)	48 (37–66.5)
ALT (IU/l)	55 (37–87)	57 (37–92)	50 (33–78)	53 (39–82.5)
γGTP (IU/l)	40 (25–72)	36 (23–65.75)	36 (23–69)	52 (32–86)a
Albumin (g/dl)	3.9 (3.7–4.1)	3.9 (3.7–4.1)	3.8 (3.7–4)	3.8 (3.7–4.1)
Total cholesterol (mg/dl)	171 (150–192)	169 (149.2–192)	175 (158–191)	170 (148.5–192.5)
Viral load (log IU/ml)	6.5 (6.1–6.9)	6.4 (5.9–6.825)	6.6 (6.3–7)	6.6 (6.2–7)
PEG-IFN-α2b (μg)	80 (80–100)	80 (80–100)	80 (75–100)	80 (60–100)
PEG-IFN-α2b/kg (μg/kg)	1.19 (1.19–1.48)	1.36 (1.19–1.48)	1.19 (1.19–1.48)	1.19 (1.02–1.48)
Ribavirin (mg)	600 (600–800)	600 (600–800)	600 (600–800)	600 (400–800)
Ribavirin/kg (mg/kg)	8.9 (8.9–11.87)	10.29 (8.9–11.87)	8.9 (8.9–11.87)	8.9 (7.8–11.86)
rs12979860 (CC/CT/TT)	582/203/27	311/51/4	128/43/4	143/109/19
rs8099917 (TT/TG/GG)	588/199/25	311/51/3	132/40/4	145/108/18

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; M, male; NVR, non-virological response; PEG-IFN, pegylated interferon; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

(56% vs 25%; OR=0.26; p=3.33E-16). Patients with the rs8099917 TT genotype had marginally higher baseline viral load (6.6 vs 6.4 log IU/ml; p=0.077) but showed a significantly greater drop in viral load by week 4 of treatment (−3.1 vs −0.8 log IU/ml; p<2.2E-16). The rs8099917 TT genotype was also associated with wild-type core70 (79% vs 56%; p= 3.1E-6) and non-wild-type ISDR (68% vs 83%; p=0.015).

Viral substitutions

Patients who achieved an SVR had significantly lower initial HCV RNA levels than those who did not (6.4 vs 6.6 log IU/ml; p=2.1E-6). The 140 patients (17%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve an SVR than patients with wild type core70 (33% vs 53%; p=0.00019) and were significantly more likely to show an NVR (42% vs 25%; p=0.0013). The 179 (22%) of patients with a substitution at position 91 (core91) were marginally less likely to achieve an SVR (41% vs 50%; p=0.08) but were significantly more likely to show an NVR (37% vs 27%; p=0.039). The 78 (10%) of patients who had two or more substitutions in the ISDR of NS5A were only marginally less likely to achieve an SVR than those with wild-type ISDR (43% vs 55%; p=0.066) and were not more likely to show an NVR (33% vs 26%; p=0.24).

Predictive factors for an SVR

Significant univariate predictors for an SVR included patient clinical factors (age, sex, diabetes, platelet count, white blood cell count, haemoglobin level, γGTP level); SNP genotype (rs12979860 and rs8099917); and viral factors (baseline viral load and core70, core91 and ISDR substitutions) (table 2). Following multivariate analysis, only age, rs12979860 genotype, core70 substitution and baseline viral load were significant independent predictors (figure 1A). The joint effects of rs12979860 and core70 on response to treatments are illustrated in figure 2.

Predictive factors for an NVR

Significant univariate predictors for an NVR included age, rs12979860 and rs8099917 genotypes, core70 and core91 substitutions, diabetes, aspartate aminotransferase (AST), baseline viral load, platelet count, white blood cell count and γGTP levels (table 3). Following multivariate analysis only age and rs12979860 genotype remained as independent predictors (figure 1B).

Predictive factors for change in viral load by week 4 of treatment

Factors influencing virological response were assessed by examining change in viral load between the start of treatment and week 4. Using linear regression, sex, rs12979860, rs8099917, core70, core91, ISDR, baseline viral load, alanine aminotransferase (ALT), platelet count, white blood cell count, haemoglobin level and γGTP were found to be significant univariate predictors of change in viral load by week 4 (table 4). Independent factors included rs12979860, core70, ISDR, ALT, platelet count and γGTP. We also found a significant positive linear relationship between the total number of ISDR substitutions and change in viral load between week 0 and week 4 (slope=0.2; p=0.0047).

In patients with the favourable rs12979860 CC genotype, core70 wild type was a significant predictor of viral decline (p=0.007; figures 3A,B), but in patients with the CT or TT genotypes, viral decline did not vary with respect to core70 substitutions (p=0.18; figures 3C,D). Conversely, ISDR was not

Table 2 Predictors for a sustained virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	0.58	1.22E-08***	362	0.432	0.31 to 0.60	6.61E-07***
Sex (male vs female)	813	1.28	0.0006***	362	1.2	0.95 to 1.54	0.133
BMI (kg/m^2)	800	0.87	0.1286				
rs12979860 (CC vs TC/TT)	812	3.65	2.67E-14***	362	4.98	2.81 to 8.82	4.00E-08***
rs8099917 (TT vs GT/GG)	812	3.53	1.77E-13***				
Hypertension	813	0.92	0.6452				
Diabetes	813	0.53	0.005907**				
Core amino acid 70 (wild type vs mutant)	395	0.42	5.82E-05***	362	0.527	0.31 to 0.89	0.01575*
Core amino acid 91 (wild type vs mutant)	399	0.66	0.0419*				
ISDR	376	1.12	0.1627				
Viral load (log IU/ml)	695	0.68	2.09E-06***	362	0.77	0.62 to 0.96	0.02249*
Fibrosis (F0–1 vs F2–4)	559	0.74	0.0817				
Activity (A0–1 vs A2–4)	546	0.96	0.7975				
Total cholesterol (mg/dl)	663	0.86	0.2151				
AST (IU/l)	687	1.03	0.1069				
ALT (IU/l)	692	1.26	0.0920				
Platelets (×10^4/L)	694	1.49	3.57E-05***	362	1.39	0.97 to 1.99	0.073
WBC (/L)	693	1.31	0.0014**				
Haemoglobin (g/dl)	693	1.28	0.0043**				
γGTP (IU/l)	646	0.96	0.0052**				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

a significant predictor of viral decline in patients with the rs12979860 CC genotype (p=0.078; figures 4A,B), but patients with the CT or TT genotypes and two or more substitutions in the ISDR showed significantly greater viral decline by week 4 than patients with zero or one ISDR substitution (p=0.007; figures 4C,D).

DISCUSSION

In this study we showed that host factors (younger age, male sex, favourable IL28B SNP genotypes) as well as viral factors (baseline viral load, wild-type core70 and two or more substitutions in the ISDR) contribute to the successful outcome of PEG-RBV combination therapy. Although some of these factors independently predict an SVR or NVR in multivariate analysis, collectively they reflect a complex genotype-by-environment interaction involving common polymorphisms in both the virus and the human host.

Genetic variation within the human IL28 locus has been reported as the strongest pretreatment predictor of an SVR,[15] and the results of this study support this finding. Several tightly linked SNPs in the non-coding region of *IL28A* and *IL28B* have been shown to be associated with spontaneous viral clearance, rapid and early virological response and/or SVR following treatment with interferon and ribavirin for HCV genotype 1b.[9–15] *IL28A*, *IL28B* and *IL29* code for type III (λ) interferons, which are similar to type I interferons but use a different receptor and show high tissue specificity.[27 28] It has not been determined which, if any, of the reported SNPs directly affects function, but the functional SNP probably affects gene expression. IRF3- and IRF7-binding sites near the transcription start

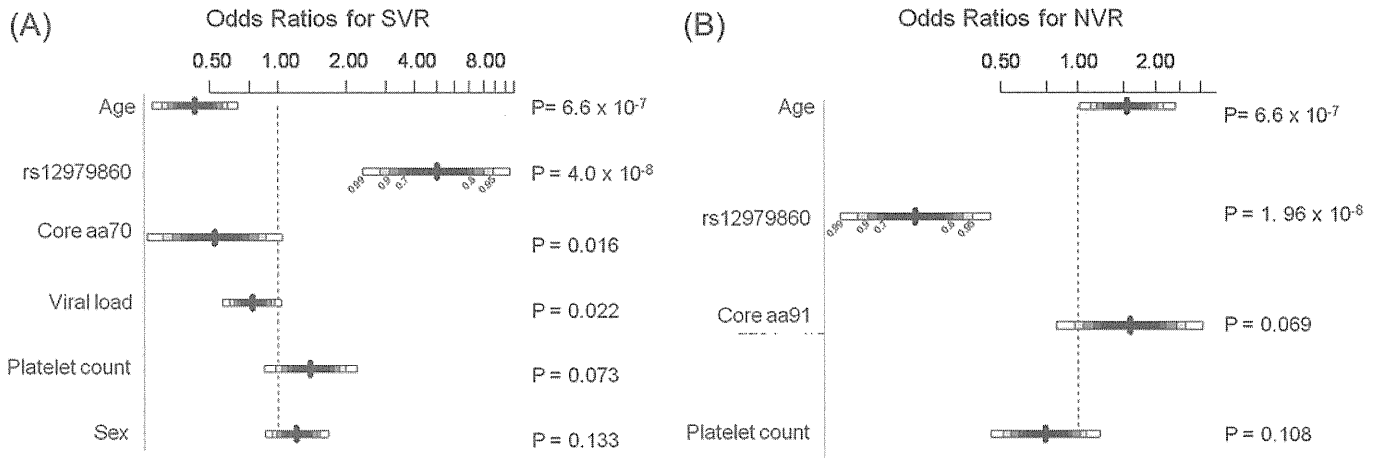

Figure 1 ORs for predictive factors response to treatment. ORs and 95% CIs are shown for predictive factors for (A) sustained virological response (SVR) and (B) non-virological response (NVR) based on multiple logistic regression with stepwise selection.

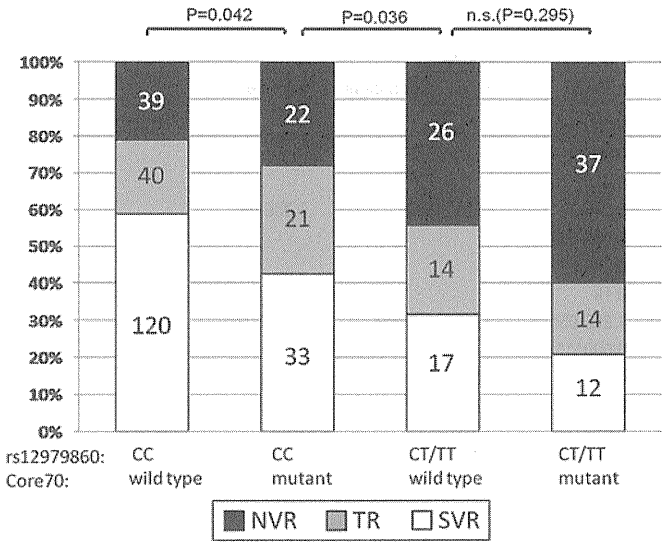

Figure 2 Cumulative effects of rs12979860 genotype and core protein amino acid 70 substitutions. The relative effects of rs12979860 genotype (favourable CC vs non-favourable CT/CC) and core amino acid 70 substitutions (favourable wild type vs unfavourable substitutions) on response to treatment are shown. NVR, non-virological response; TR, transient response/relapser; SVR, sustained virological response.

site of *IL28B* are essential for gene expression, but distal clusters of nuclear factor-κB (NF-κB)-binding sites are necessary for maximal expression,[29 30] suggesting that upstream polymorphisms may potentially disrupt transcription factor-binding sites within a distal promoter or enhancer. Unintuitively, interferon-stimulated genes are downregulated in patients with the favourable rs8099917 TT genotype,[31] implying that responders have a lower baseline expression of immune response genes.[32] This might serve to prevent desensitisation and promote maximal induction of interferon-stimulated genes, but detailed gene regulation studies are needed to resolve the role of IL28B polymorphisms in antiviral defence.

In addition to effects of human genetic polymorphisms, a number of studies have reported significant association between HCV core70/core91 substitutions and treatment outcome.[20 33 34] We found significant independent associations between core70 substitutions and an SVR, as well as change in viral load by week 4, but the association was not significant for an NVR under multivariate analysis despite being highly significant in univariate analysis. Although the role of core70 substitutions is unclear, the core protein interacts with a number of viral and host proteins and disrupts the interferon signalling pathway.[35–37] The proportion of core70 substitutions in the host viral population has been reported to increase during treatment with PEG-RBV therapy, which may indicate positive selection at this position in response to treatment.[38] Substitutions at these positions appear to affect the antiviral response during the early stages of treatment, as wild-type core70 and core91 are associated with a rapid decrease in HCV RNA levels during the first 4 weeks of treatment.[39 40] Because a rapid virological response is also a strong predictor of SVR and NVR, core70 and core91 substitutions may affect treatment outcome either directly or indirectly.[40 41]

Unlike HCV core70 substitutions, we found only a marginal association between ISDR substitutions and SVR, and no association with NVR. However, ISDR substitution was a significant independent predictor of change in viral load by week 4. The presence of two or more mutations in this 40 amino acid stretch of the NS5A protein is associated with an SVR.[24 42] Other studies have found no significant association between ISDR and SVR but have found a higher overall mutation rate in the NS5A protein among SVR patients,[43 44] and one study suggests that the association with ISDR varies by strain and is more pronounced in Japan than in Europe.[45] It is not clear whether mutations in ISDR directly affect function or whether they reflect the genetic distance from an interferon-resistant

Table 3 Predictors for a non-virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	1.30	0.01306*	370	1.55	1.12 to 2.15	0.008367**
Sex (male vs female)	813	0.90	0.178				
BMI (kg/m^2)	800	1.07	0.3899				
rs12979860 (CC vs TC/TT)	812	0.26	2.73E-17***	370	0.231	0.14 to 0.39	1.96E-08***
rs8099917 (TT vs GT/GG)	812	0.26	1.51E-17***				
Hypertension	813	1.16	0.4323				
Diabetes	813	1.55	0.04685*				
Core amino acid 70 (wild type vs mutant)	395	2.17	0.000496***				
Core amino acid 91 (wild type vs mutant)	399	1.66	0.02029*	370	1.58	0.96 to 2.60	0.06943
ISDR	376	0.92	0.06197				
Viral load (log IU/ml)	695	1.32	0.01716*				
Fibrosis (F0–1 vs F2–4)	559	1.24	0.2608				
Activity (A0–1 vs A2–4)	546	1.12	0.5499				
Total cholesterol (mg/dl)	663	0.98	0.5824				
AST (IU/l)	687	1.02	0.03148*				
ALT (IU/l)	692	0.91	0.8772				
Platelets (×10^4/L)	694	0.76	0.008222**	370	0.739	0.51 to 1.07	0.1077
WBC (/L)	693	0.83	0.04617*				
Haemoglobin (g/dl)	693	0.84	0.1201				
γGTP (IU/l)	646	1.15	1.23E-05***				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

Table 4 Predictors for change in viral load by week 4 of treatment

	Simple			Multiple		
Variable	**n**	**Coefficient**	**p Value**	**n**	**Coefficient**	**p Value**
Age	500	−0.01	0.138			
Sex (male vs female)	500	−0.23	0.005**			
BMI (kg/m^2)	494	0.00	0.958			
rs12979860 (CC vs TC/TT)	500	2.11	5.18E-38***	221	1.37	1.35E-08***
rs8099917 (TT vs GT/GG)	499	2.10	1.40E-36***			
Hypertension	500	−0.25	0.249			
Diabetes	500	−0.31	0.19			
Core amino acid 70 (wild type vs mutant)	259	−1.01	1.38E-05***	221	−0.665	0.001328**
Core amino acid 91 (wild type vs mutant)	262	−0.77	0.000***			
ISDR	247	0.20	0.006**	221	0.186	0.001878**
Viral load (log IU/ml)	500	0.37	0.000***	221	0.414	0.00012***
Fibrosis (F0−1 vs F2−4)	397	−0.22	0.217			
Activity (A0−1 vs A2−4)	389	−0.10	0.578			
Total cholesterol (mg/dl)	472	0.00	0.064			
AST (IU/l)	490	0.00	0.442			
ALT (IU/l)	493	0.00	0.005**	221	0.00606	0.008895**
Platelets (×10^4/L)	495	0.03	0.048*	221	0.0701	7.24E-05***
WBC (/L)	495	0.00	0.027*			
Haemoglobin (g/dl)	495	0.13	0.013*			
γGTP (IU/l)	460	0.00	0.001***	221	−0.00634	0.002095**

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

strain. Nonetheless, the NS5A protein has been shown to be under purifying selection[44] and plays a critical role in both viral replication[46 47] and modulation of the immune response.[48] Therefore, the number of substitutions in one or more variable regions of the NS5A may be a useful predictor of early viral dynamics and an indirect predictor of SVR, although in this study we found a significant effect only for change in viral load by week 4 of treatment.

A number of factors have now been reported to influence outcome of PEG-RBV therapy, and it is important to determine which of these factors represent independent, clinically useful predictors. Because of the expense and occasionally severe side effects of the current standard of care, reliable pretreatment indicators, especially of poor response, will help guide treatment decisions and steer difficult-to-treat patients towards more

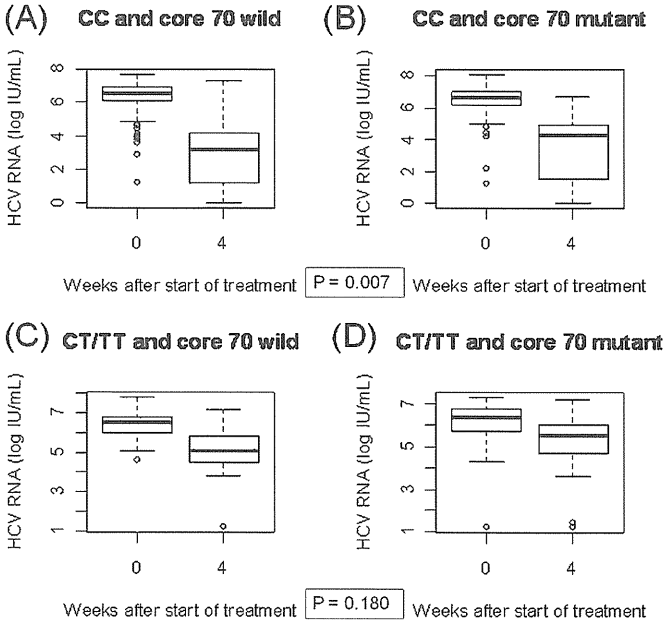

Figure 3 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and hepapitis C virus (HCV) core protein substitutions. The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and wild/mutant amino acid at core70 is shown.

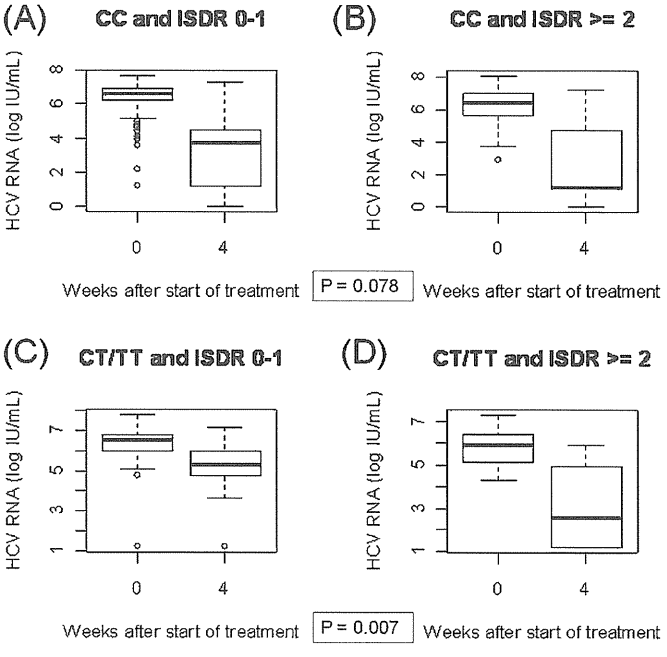

Figure 4 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and substitutions in the interferon sensitivity-determining region (ISDR). The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and the number of substitutions in the ISDR is shown.

Gut 2011;**60**:261−267. doi:10.1136/gut.2010.223495

effective treatments or enrolment in clinical trials. In order to identify the most important independent predictors, it will be necessary to disentangle the intriguing interactions between human and viral polymorphisms as well as gain better understanding of the role of type III interferon in the immune response against HCV.

Acknowledgements We thank Mika Tsuzuno, Sakura Akamatsu, Sanae Furuya and other members of the clerical staff, and Yoshiiku Kawakami and other physicians at Hiroshima University Hospital for their help.

Funding This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare, Government of Japan.

Competing interests None.

Ethics approval This study was conducted with the approval of the Hiroshima University ethics committee.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Alter MJ.** Epidemiology of hepatitis C in the West. *Semin Liver Dis* 1995;**15**:5—14.
2. **Chevaliez S,** Pawlotsky JM. Hepatitis C virus: virology, diagnosis and management of antiviral therapy. *World J Gastroenterol* 2007;**13**:2461—6.
3. **Hadziyannis SJ,** Sette H Jr, Morgan TR, *et al*. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;**140**:346—55.
4. **Hijikata M,** Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. *Intervirology* 2000;**43**:124—7.
5. **Knapp S,** Yee LJ, Frodsham AJ, *et al*. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. *Genes Immun* 2003;**4**:411—19.
6. **Matsuyama N,** Mishiro S, Sugimoto M, *et al*. The dinucleotide microsatellite polymorphism of the IFNAR1 gene promoter correlates with responsiveness of hepatitis C patients to interferon. *Hepatol Res* 2003;**25**:221—5.
7. **Naito M,** Matsui A, Inao M, *et al*. SNPs in the promoter region of the osteopontin gene as a marker predicting the efficacy of interferon-based therapies in patients with chronic hepatitis C. *J Gastroenterol* 2005;**40**:381—8.
8. **Tsukada H,** Ochi H, Maekawa T, *et al*. A polymorphism in MAPKAPK3 affects response to interferon therapy for chronic hepatitis C. *Gastroenterology* 2009;**136**:1796—805, e6.
9. **Ge DL,** Fellay J, Thompson AJ, *et al*. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**:399—401.
10. **Tanaka Y,** Nishida N, Sugiyama M, *et al*. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;**41**:1105—9.
11. **Suppiah V,** Moldovan M, Ahlenstiel G, *et al*. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nature Genetics* 2009;**41**:1100—4.
12. **Thomas DL,** Thio CL, Martin MP, *et al*. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009;**461**:798—801.
13. **Rauch A,** Kutalik Z, Descombes P, *et al*. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010;**138**:1338—45, e1—7.
14. **McCarthy JJ,** Li JH, Thompson A, *et al*. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010;**138**:2307—14.
15. **Thompson AJ,** Muir AJ, Sulkowski MS, *et al*. IL28B polymorphism improves viral kinetics and is the strongest pre-treatment predictor of SVR in HCV-1 patients. *Gastroenterology* 2010;**139**:120—9.
16. **Zeuzem S,** Franke A, Lee JH, *et al*. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. *Hepatology* 1996;**24**:1003—9.
17. **Kau A,** Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. *J Hepatol* 2008;**49**:634—51.
18. **Adinolfi LE,** Utili R, Andreana A, *et al*. Relationship between genotypes of hepatitis C virus and histopathological manifestations in chronic hepatitis C patients. *Eur J Gastroenterol Hepatol* 2000;**12**:299—304.
19. **Enomoto N,** Sakuma I, Asahina Y, *et al*. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis-C virus 1b—sensitivity to interferon is conferred by amino-acid substitutions in the NS5A region. *J Clin Invest* 1995;**96**:224—30.
20. **Akuta N,** Suzuki F, Sezaki H, *et al*. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon—ribavirin combination therapy. *Intervirology* 2005;**48**:372—80.
21. **Desmet VJ,** Gerber M, Hoofnagle JH, *et al*. Classification of chronic hepatitis—diagnosis, grading and staging. *Hepatology* 1994;**19**:1513—20.
22. **Akuta N,** Suzuki F, Kawamura Y, *et al*. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007;**46**:403—10.
23. **Akuta N,** Suzuki F, Sezaki H, *et al*. Predictive factors of virological non-response to interferon—ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 2006;**78**:83—90.
24. **Enomoto N,** Sakuma I, Asahina Y, *et al*. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996;**334**:77—81.
25. **Ohnishi Y,** Tanaka T, Ozaki K, *et al*. A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet* 2001;**46**:471—7.
26. **Suzuki A,** Yamada R, Chang X, *et al*. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003;**34**:395—402.
27. **Kotenko SV,** Gallagher G, Baurin VV, *et al*. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 2003;**4**:69—77.
28. **Marcello T,** Grakoui A, Barba-Spaeth G, *et al*. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology* 2006;**131**:1887—98.
29. **Thomson SJ,** Goh FG, Banks H, *et al*. The role of transposable elements in the regulation of IFN-lambda1 gene expression. *Proc Natl Acad Sci USA* 2009;**106**:11564—9.
30. **Iversen MB,** Ank N, Melchjorsen J, *et al*. Expression of type III interferon (IFN) in the vaginal mucosa is mediated primarily by dendritic cells and displays stronger dependence on NF-kappaB than type I IFNs. *J Virol* 2010;**84**:4579—86.
31. **Honda M,** Sakai A, Yamashita T, *et al*. Hepatic ISG expression is associated with genetic variation in IL28B and the outcome of IFN therapy for chronic hepatitis C. *Gastroenterology* 2010;**139**:499—509.
32. **Li JH,** Lao XQ, Tillmann HL, *et al*. Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. *Hepatology* 2010;**51**:1904—11.
33. **Akuta N,** Suzuki F, Kawamura Y, *et al*. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2007;**79**:1686—95.
34. **Akuta N,** Suzuki F, Hirakawa M, *et al*. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 2a high viral load and virological response to interferon-ribavirin combination therapy. *Intervirology* 2009;**52**:301—9.
35. **de Chassey B,** Navratil V, Tafforeau L, *et al*. Hepatitis C virus infection protein network. *Mol Syst Biol* 2008;**4**:230.
36. **Ciccaglione AR,** Stellacci E, Marcantonio C, *et al*. Repression of interferon regulatory factor 1 by hepatitis C virus core protein results in inhibition of antiviral and immunomodulatory genes. *J Virol* 2007;**81**:202—14.
37. **Luquin E,** Larrea E, Civeira MP, *et al*. HCV structural proteins interfere with interferon-alpha Jak/STAT signalling pathway. *Antiviral Res* 2007;**76**:194—7.
38. **Kurbanov F,** Tanaka Y, Matsuura K, *et al*. Positive selection of core 70Q variant genotype 1b hepatitis C virus strains induced by pegylated interferon and ribavirin. *J Infect Dis* 2010;**201**:1663—71.
39. **Akuta N,** Suzuki F, Hirakawa M, *et al*. Amino acid substitutions in the hepatitis C virus core region of genotype 1b affect very early viral dynamics during treatment with telaprevir, peginterferon, and ribavirin. *J Med Virol* 2010;**82**:575—82.
40. **Hayashi K,** Katano Y, Ishigami M, *et al*. Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. *J Viral Hepat* 2010; Epub ahead of print.
41. **Ishii K,** Shinohara M, Sawa M, *et al*. Interferon alpha receptor 2 expression by peripheral blood monocytes in patients with a high viral load of hepatitis C virus genotype 1 showing substitution of amino acid 70 in the core region. *Intervirology* 2010;**53**:105—10.
42. **Nakagawa M,** Sakamoto N, Ueyama M, *et al*. Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. *J Gastroenterol* 2010;**45**:656—65.
43. **Jardim AC,** Yamasaki LH, de Queiroz AT, *et al*. Quasispecies of hepatitis C virus genotype 1 and treatment outcome with peginterferon and ribavirin. *Infect Genet Evol* 2009;**9**:689—98.
44. **Bittar C,** Jardim AC, Yamasaki LH, *et al*. Genetic diversity of NS5A protein from hepatitis C virus genotype 3a and its relationship to therapy response. *BMC Infect Dis* 2010;**10**:36.
45. **Pascu M,** Martus P, Hohne M, *et al*. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004;**53**:1345—51.
46. **Masaki T,** Suzuki R, Murakami K, *et al*. Interaction of hepatitis C virus nonstructural protein 5A with core protein is critical for the production of infectious virus particles. *J Virol* 2008;**82**:7964—76.
47. **Gao M,** Nettles RE, Belema M, *et al*. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010;**465**:96—100.
48. **Pang PS,** Planet PJ, Glenn JS. The evolution of the major hepatitis C genotypes correlates with clinical response to interferon therapy. *PLoS One* 2009;**4**:e6579.

Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

Fumitaka Suzuki,[1] Yoshiyuki Suzuki,[1] Norio Akuta,[1] Hitomi Sezaki,[1] Miharu Hirakawa,[1] Yusuke Kawamura,[1] Tetsuya Hosaka,[1] Masahiro Kobayashi,[1] Satoshi Saito,[1] Yasuji Arase,[1] Kenji Ikeda,[1] Mariko Kobayashi,[2] Kazuaki Chayama,[3] Naoyuki Kamatani,[4] Yusuke Nakamura,[5] Yuzo Miyakawa,[6] and Hiromitsu Kumada[1]

Polymorphisms of the inosine triphosphatase (*ITPA*) gene influence anemia during pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy, but their effects during triple therapy with PEG-IFN, RBV, and telaprevir are not known. Triple therapy for 12 weeks, followed by PEG-IFN and RBV for 12 weeks, was given to 49 patients with RBV-sensitive (CC at rs1127354) and 12 with RBV-resistant (CA/AA) *ITPA* genotypes who had been infected with hepatitis C virus (HCV) of genotype 1. Decreases in hemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$), as well as at the end of treatment (−2.9 ± 1.1 vs. −2.0 ± 0.86, $P = 0.013$). Risk factors for hemoglobin <11.0 g/dL at week 4 were female gender, age >50 years, body mass index (BMI) <23, and CC at rs1127354 by multivariate analysis. RBV dose during the first 12 weeks was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$), but the total RBV dose was no different between them (49 ± 17% and 54 ± 18% of the target, $P = 0.531$). Sustained virological response (SVR) was achieved in 70% and 64% of them, respectively ($P = 0.724$). *Conclusion: ITPA* polymorphism influences hemoglobin levels during triple therapy, particularly during the first 12 weeks while telaprevir is given. With careful monitoring of anemia and prompt adjustment of RBV dose, SVR can be achieved comparably frequently between patients with CC and CA/AA genotypes. (HEPATOLOGY 2011;53:415-421)

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28B, *interleukin 28B;* ITPA, *inosine triphosphatase; PEG-IFN, pegylated interferon; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response.*

From the [1]Department of Hepatology, Toranomon Hospital, Tokyo, Japan; [2]Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan; [3]Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan; [4]Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan; [5]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [6]Miyakawa Memorial Research Foundation, Tokyo, Japan.

Received August 12, 2010; accepted October 1, 2010.

Supported in part by grants from the Japanese Ministry of Health, Labour and Welfare.

Address reprint requests to: Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. E-mail: fumitakas@toranomon.gr.jp; fax: +81-44-860-1623.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24058

Potential conflict of interest: Nothing to report.

Worldwide, 123 million people are estimated to have been infected with hepatitis C virus (HCV),[1] and ≈30% of them develop fatal liver disease such as cirrhosis and hepatocellular carcinoma.[2,3] Currently, the standard of care therapy for patients infected with HCV is pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks.[4-6] However, the combined treatment can induce a sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after treatment completion, in at most 50% of patients infected with HCV-1, the genotype most prevalent and least responsive to IFN-based therapies.

Recently, Fellay et al.[7] reported that polymorphisms of the inosine triphosphatase (*ITPA*) gene in chromosome 20 (20p13) influence RBV-induced anemia in a genome-wide association study (GWAS). Single nucleotide polymorphism (SNP) at rs1127354 for proline-to-threonine substitution (P32T) in the second of eight

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of ITPA.[8-11] Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.[7] Their observations have been extended to many patients in a large-scale trial with pegIFN-α-2a on Caucasian and African Americans,[12] as well as in the Japanese receiving PEG-IFN-α-2b and RBV who were infected with HCV-1.[13]

For improving SVR in HCV-1 patients, protease inhibitors have been added to the standard treatment with PEG-IFN and RBV, and increased SVR by ≈20%.[14-16] However, such a gain in efficacy is not without trade-offs, represented by aggravation of anemia. Early decreases in hemoglobin levels during the triple therapy reach 4 g/dL, and they exceed ≈3.0 g/dL in the standard treatment.[14,15] Because there have been no reports focusing on the influence of *ITPA* genotypes on anemia developing in patients during triple therapy, hemoglobin levels were followed in 61 Japanese patients with HCV-1 who had received it. The results were correlated with polymorphisms at rs1127354 in the *ITPA* gene because the Japanese are monoallelic at rs7270101 and have the AA genotype exclusively.[11]

Patients and Methods

Study Cohort. This retrospective cohort study was performed in 61 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria were: (1) diagnosed with chronic hepatitis C; (2) HCV-1 confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels ≥5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (4) Japanese aged from 20 to 65 years at the entry; and (5) body weight between ≥40 kg and ≤120 kg at the time of registration. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) hepatitis B surface antigen in serum; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance ≤50 mL/min at the baseline; (6) hemoglobin ≤12 g/dL, neutrophil ≤1,500/mm^3 or platelet ≤100,000/mm^3 at baseline.

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

Triple Treatment with PEG-IFN-α-2b, RBV, and Telaprevir. Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan), 750 mg, was administered 3 times a day at an 8-hour (q8) interval after each meal. Pegylated-IFN-α-2b (PEG-Intron, Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.32-1.71 μg/kg) once a week. RBV (Rebetol, Schering Plough) 200-600 mg was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ≈≤80 kg; and 1,000 mg for ≥80 kg. The triple therapy with PEG-IFN-α-2b, RBV, and telaprevir was continued for 12 weeks, and then switched to PEG-IFN-α-2b and RBV for an additional 12 weeks. It was withdrawn when hemoglobin levels decreased <8.5 g/dL. After the therapy was completed or discontinued, patients were followed for 24 weeks for SVR.

The RBV dose was cut by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1,000 mg) when hemoglobin decreased <12 g/dL, and by another 200 mg when it was below <10 g/dL. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dL at baseline and those in whom it decreased by 1 g/dL to <13 g/dL within a week. PEG-IFN dose was reduced by one-half when the leukocyte count decreased <1,500/mm^3, neutrophil count <750/mm^3, or platelet count <80 × 10^3/mm^3; PEG-IFN was withdrawn when they decreased <1,000/mm^3, 500/mm^3, or 50 × 10^3/mm^3, respectively.

The triple therapy was withdrawn or stopped temporarily when hemoglobin decreased <8.5 g/dL. In patients in whom hemoglobin increased ≥8.5 g/dL within 2 weeks after the withdrawal, treatment was resumed with PEG-IFN and RBV 200 mg. A reduction of telaprevir (MP-424) dose was not permitted. It was discontinued when severe side effects appeared, whereas PEG-IFN and RBV were continued. Growth factors were not used for elevating hemoglobin levels.

Determination of ITPA Genotypes. *ITPA* (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described.[17,18]

Statistical Analyses. Continuous variables between groups were compared by the Mann-Whitney test (*U* test), and discontinuous variables by the chi-square test

Table 1. Baseline Characteristics of the 61 Patients Infected with HCV-1 Who Received Triple Therapy with Pegylated-Interferon, Ribavirin, and Telaprevir

		ITPA Genotypes at rs1127354	
	Total	CC	CA + AA
Demographic data			
Number	61	49	12
Sex (male/female)	34/27	28/21	6/6
Age (years)	56 (23-65)	55 (23-65)	58 (28-62)
Body weight (kg)	61.5 (41.0-92.9)	61.5 (41.0-92.9)	62.1 (44.4-81.1)
Body mass index (kg/m^2)	22.6 (17.6-32.4)	22.2 (17.6-32.4)	22.9 (17.8-26.5)
Genotypes of the *IL28B* gene			
rs8099917 (for 59 patients)			
(TT/TG + GG)	33/26	27/21	6/7
rs12979860 (for 57 patients)			
(CC/CT + TT)	30/27	36/22	4/5
Laboratory data			
Hemoglobin (g/dL)	14.4 (12.5-16.6)	14.4 (12.5-16.6)	14.2 (12.8-16.3)
Platelets (x 10^4/mm^3)	17.8 (9.1-33.8)	17.7 (9.1-33.8)	19.5 (13.1-31.6)
Albumin (g/dL)	3.9 (3.2-4.6)	3.9 (3.2-4.6)	3.9 (3.5-4.1)
Alanine aminotransferase (U/L)	39 (12-175)	41 (12-175)	28 (17-57)
Aspartate aminotransferase (U/L)	32 (15-137)	35 (15-137)	28 (20-35)
HCV RNA (log IU/mL)	6.7 (5.1-7.6)	6.8 (5.7-7.6)	6.6 (5.1-7.5)
HCV genotype 1a/1b	1/60	1/48	0/12
Previous IFN-based treatment			
Treatment naïve	17	12 (24%)	5 (42%)
Relapsed	29	23 (47%)	6 (50%)
Null response	15	14 (29%)	1 (8%)

Data are median values (range) or n.

and Fisher's exact test. Kaplan-Meier analysis and the log-rank test were applied to estimate and compare decreases of RBV dose between groups. Factors evaluated for influence on hemoglobin decrease by univariate analysis were: sex; age; body mass index (BMI); body weight; hemoglobin levels; initial PEG-IFN and RBV doses; amino acid substitutions in the HCV core protein; number of amino acid substitutions in the interferon sensitivity determining region; and *IL28B* polymorphisms (at rs8099917 and rs12979860). Factors associated with a decrease in hemoglobin levels ($P < 0.10$) were assessed by multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence interval (CI) was determined. All analyses were performed using SPSS software (SPSS II v. 11.0, Chicago, IL), and a P-value < 0.05 was considered significant.

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (<13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

Modification of RBV Dose During Triple Therapy. RBV dose was reduced by ≥200 mg in all 61 patients studied during triple therapy because hemoglobin had decreased <12.0 g/dL in them. During the first 12 weeks of therapy while telaprevir was given, the proportion of patients receiving the full RBV dose differed between those with CC and CA/AA genotypes (Fig. 1). RBV dose reduction was started earlier in the 49 patients with CC than the 12 with CA/AA genotypes (2.6 ± 1.3 vs. 4.8 ± 3.1 weeks after the start, respectively, $P = 0.010$). Thus, during the first 12 weeks with telaprevir the RBV dose was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the next 12

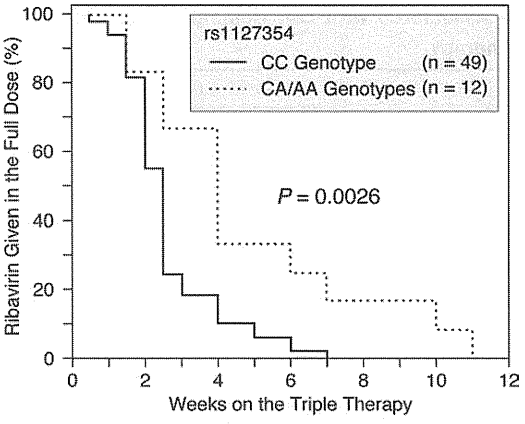

Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

weeks without telaprevir, in contrast, the RBV dose was somewhat larger in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). The total RBV dose during 24 weeks on therapy was comparable between the 49 patients with CC and the 12 with CA/AA genotypes (49 ± 17% vs. 54 ± 18%, $P = 0.531$). In patients with the CC genotype, the RBV dose was no different between those who achieved SVR and those who did not (50 ± 18% vs. 47 ± 13%, $P = 0.728$). The RBV dose did not differ either in patients with CA/AA genotypes with and without SVR (57 ± 17% vs. 48 ± 20%, $P = 0.368$).

The total dose of PEG-IFN was comparable among 49 patients with CC and 12 with CA/AA genotypes (87 ± 23% vs. 86 ± 20% of the target, $P = 0.488$). The total telaprevir dose was no different either between them (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$). Telaprevir was discontinued in 10 of the 49 (20%) patients with CC and 5 of the 12 (42%) with CA/AA genotypes ($P = 0.147$).

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4-10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately −4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and were significant at week 20 (−3.0 ± 1.2 vs. −2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (−2.9 ± 1.1 vs. −2.0 ± 0.85 g/dL, $P = 0.013$).

SVR was achieved by 35 (71%) of the 49 patients with CC and 8 (67%) of the 12 with CA/AA genotypes ($P = 0.736$). Hemoglobin levels did not differ between them 24 weeks after the completion of triple therapy (−0.57 ± 1.1 vs. −0.17 ± 0.87 g/dL, $P = 0.271$). Of the 32 patients with TT genotype of the *IL28B* gene at rs8099917, 30 (94%) gained SVR, more frequently than 10 of the 26 (38%) with TG/GG genotypes ($P < 0.001$). Likewise, 29 of the 30 (97%) patients with CC genotype at rs12979860 achieved SVR, more frequently than 11 of the 27 (41%) with CT/TT genotypes ($P < 0.001$).

Factors Influencing Decreases in Hemoglobin Levels. Hemoglobin decreased <11 g/dL at week 4 during the triple therapy in 27 of the 61 (44%) patients. Factors for hemoglobin <11.0 g/dL were female gender, age >50 years, body weight <60 kg, BMI <23, and baseline hemoglobin <15 g/dL, as well as the CC genotype of the *ITPA* gene, in the univariate analysis (Table 2). Of them, female gender, age >50 years, BMI <23, and the CC genotype remained significant in the multivariate analysis. Hemoglobin levels lowered <8.5 g/dL during the triple therapy in 13 of the 61 (21%) patients. Factors for hemoglobin <8.5 g/dL were female gender, age >60 years, body weight <60 kg, BMI <23, and baseline hemoglobin <14 g/dL in the univariate analysis (Table 3). Of

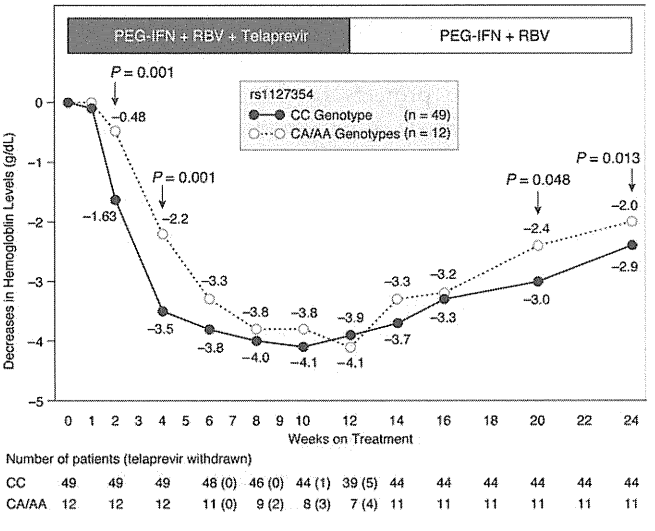

Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN and RBV continued) in parentheses.

Table 2. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Low Hemoglobin Levels (< 11.0 g/dL) at Week 4 of Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	14.3 (4.1-50.0)	< 0.001	29.41 (3.8-250.0)	0.001
Age (> 50 years)	4.3 (1.0-17.5)	0.030	7.3 (1.1-47.6)	0.039
Body weight (< 60 kg)	11.5 (3.4-38.2)	< 0.001		
Body mass index (< 23)	8.4 (2.6-27.1)	< 0.001	17.2 (2.6-112.0)	0.003
Hemoglobin (< 15g/dL)	14.2 (3.5-57.4)	< 0.001		
ITPA gene (CC genotype)		0.062	36.8 (2.5-550.2)	0.009

Abbreviations: OR, odds ratio; CI, confidence level.

them, only age and body weight remained significant in the multivariate analysis.

Discussion

Anemia is a substantial risk in the standard of care therapy with PEG-IFN and RBV.[4-6] Triphosphorylated RBV accumulates in erythrocytes of patients who receive RBV, increasingly with RBV dose and duration, and causes oxidative damage to erythrocyte membranes toward extravascular hemolysis by the reticuloendothelial system.[19,20] Inosine triphosphate accumulates also in erythrocytes of individuals who have mutations in the *ITPA* gene, and results in benign red-cell enzymopathy.[8] The expression of *ITPA* is genetically controlled and reduced in individuals who have point mutations in the *ITPA* gene.[8-11] As another achievement of GWAS in hepatology,[21] in the wake of polymorphisms of the *IL28B* gene that influence the response to PEG-IFN and RBV,[22-24] polymorphisms in the *ITPA* gene has been reported to influence anemia caused by RBV.[7] How inosine triphosphate protects erythrocytes from hemolysis caused by RBV needs to be sorted out by *in vivo* and *in vitro* experiments. Inosine triphosphate may prohibit the accumulation of RBV in erythrocytes, or rather, it might act directly toward prohibition of hemolysis.

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,[7] have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.[7,12] Because the Japanese are monoallelic at rs7270107 and possess the AA genotype exclusively,[11,25] only polymorphisms at rs1127354 were examined.

Of the 61 patients, 49 possessed the RBV-sensitive CC genotype and the remaining 12 had RBV-resistant CA/AA genotypes. Hemoglobin levels decreased both in patients with CC and CA/AA genotypes. They lowered ≈4 g/dL during weeks 8-12 on the triple therapy with telaprevir, and increased thereafter (Fig. 2). Between the two groups of patients, differences in hemoglobin decrease were greatest at week 4 (1.3 g/dL), as in the standard treatment with PEG-IFN and RBV.[7,12,13]

When anemia and other side effects occurred, doses of RBV, PEG-IFN, and telaprevir were modified. Of the 61 patients studied, 27 (44%) were women and most of them were in old age. Beyond 50 years of age, women are less responsive than men to the standard treatment with PEG-IFN and RBV, probably because estrogens with an antifibrotic potential decrease after menopause.[26] Stringent precautions had to be taken, therefore, by reducing the RBV dose in the patients in whom hemoglobin levels decreased <12 g/dL, rather than the conventional threshold of <10 g/dL.

Reductions of RBV dose due to anemia in patients who receive PEG-IFN and RBV are influenced by *ITPA* polymorphisms.[12] Also, in patients who had received the triple therapy the RBV dose had to be reduced more in

Table 3. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Very Low Hemoglobin Levels (<8.5 g/dL) During Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	6.1 (1.5-25.1)	0.007		
Age (>60 years)	6.8 (1.8-26.0)	0.004	10.1 (1.9-53.9)	0.007
Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
Hemoglobin (<14 g/dL)	4.3 (1.2-15.6)	0.023		

Abbreviations: OR, odds ratio; CI, confidence level.

patients with CC than CA/AA genotypes during the first 12 weeks while they received telaprevir (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the second 12 weeks off telaprevir, the RBV dose was somewhat greater in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). Thus, the total RBV dose during 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes (51 ± 15% and 57 ± 18%, $P = 0.724$). Likewise, the total dose of PEG-IFN (87 ± 23% vs. 86 ± 20% of the target, $P = 0.806$), as well as that of telaprevir (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$), was no different between patients with CC and CA/AA genotypes. SVR was achieved comparably frequently in them (71% vs. 67%, $P = 0.736$).

Decreases in hemoglobin levels during the first 12 week were similar between the current triple therapy cohort and previous patients receiving PEG-IFN and RBV.[12,13] The conservative hemoglobin levels chosen for RBV dose reduction may be a possible confounding factor on the impact of *ITPA* variants in anemia, which would have been greater should the RBV dose not be reduced in patients with RBV-sensitive CC genotypes.

ITPA polymorphisms at rs1127354 were associated with RBV-induced anemia in Japanese patients, without involvement of those at rs7270107 reported in Caucasian and African-American patients.[13] Thus, *ITPA* polymorphisms at rs1127354 would play a major role in protecting patients from RBV-induced anemia. CC/CA genotypes at rs1127354 occurs in 6% of the Caucasian population, much less often in the Oriental population, at 16%.[25,27] Although AC/CC genotypes at rs7270107 occurs in 13% of Caucasians, they do not exist in Orientals.[11,25] Obviously, different polymorphisms need to be examined in patients of distinct ethnicities when the influence on RBV-induced anemia is to be evaluated.

In confirmation of our previous report,[28] the triple therapy achieved SVR more frequently in patients with CC than CT/TT genotypes of *IL28* at rs12979860 (96% vs. 41%, $P < 0.001$). About two-thirds of studied patients accomplished SVR with the triple treatment, although one-fourth of them were nonresponders to previous IFN-based treatments; they are known to respond poorly to repeated treatments. This would lend further support to the efficacy of triple therapy being higher than treatment with pegylated IFN and RBV.

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558-567.
2. Hoofnagle JH. Course and outcome of hepatitis C. HEPATOLOGY 2002; 36:S21-29.
3. Seeff LB. Natural history of chronic hepatitis C. HEPATOLOGY 2002;36: S35-46.
4. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.
5. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-965.
6. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580-593.
7. Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature 2010;464:405-408.
8. Arenas M, Duley J, Sumi S, Sanderson J, Marinaki A. The ITPA c.94C>A and g.IVS2+21A>C sequence variants contribute to missplicing of the ITPA gene. Biochim Biophys Acta 2007;1772:96-102.
9. Cao H, Hegele RA. DNA polymorphisms in ITPA including basis of inosine triphosphatase deficiency. J Hum Genet 2002;47:620-622.
10. Stepchenkova EI, Tarakhovskaya ER, Spitler K, Frahm C, Menezes MR, Simone PD, et al. Functional study of the P32T ITPA variant associated with drug sensitivity in humans. J Mol Biol 2009;392: 602-613.
11. Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowale-Bakre M, Rees DC, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet 2002;111:360-367.
12. Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology 2010;139:1181-1189.
13. Ochi H, Mackawa T, Abe H, Hayashida Y, Nakano R, Kubo M, et al. ITPA polymorphism affects ribavirin-induced anemia and outcome of therapy — a genome-wide study of Japanese HCV patients. Gastroenterology 2010;139:1190-1197.
14. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839-1850.
15. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827-1838.

16. Mederacke I, Wedemeyer H, Manns MP. Boceprevir, an NS3 serine protease inhibitor of hepatitis C virus, for the treatment of HCV infection. Curr Opin Investig Drugs 2009;10:181-189.
17. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 2001;46:471-477.
18. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395-402.
19. De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. HEPATOLOGY 2000;31:997-1004.
20. Russmann S, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. Curr Med Chem 2006;13:3351-3357.
21. Karlsen TH, Melum E, Franke A. The utility of genome-wide association studies in hepatology. HEPATOLOGY 2010;51:1833-1842.
22. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399-401.
23. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100-1104.
24. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105-1109.
25. Maeda T, Sumi S, Ueta A, Ohkubo Y, Ito T, Marinaki AM, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. Mol Genet Metab 2005;85:271-279.
26. Sezaki H, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, et al. Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci 2009;54:1317-1324.
27. Atanasova S, Shipkova M, Svinarov D, Mladenova A, Genova M, Wieland E, et al. Analysis of ITPA phenotype-genotype correlation in the Bulgarian population revealed a novel gene variant in exon 6. Ther Drug Monit 2007;29:6-10.
28. Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. HEPATOLOGY 2010;52:421-429.

MAJOR ARTICLE

IL28B But Not ITPA Polymorphism Is Predictive of Response to Pegylated Interferon, Ribavirin, and Telaprevir Triple Therapy in Patients With Genotype 1 Hepatitis C

Kazuaki Chayama,[1,2,3] C. Nelson Hayes,[1,2,3] Hiromi Abe,[1,2,3] Daiki Miki,[1,2,3] Hidenori Ochi,[1,2,3] Yoshiyasu Karino,[4] Joji Toyota,[4] Yusuke Nakamura,[5] Naoyuki Kamatani,[7] Hitomi Sezaki,[6] Mariko Kobayashi,[6] Norio Akuta,[6] Fumitaka Suzuki,[6] and Hiromitsu Kumada[6]

[1]Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN; [2]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University; [3]Liver Research Project Center, Hiroshima University; [4]Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido; [5]Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo; [6]Department of Hepatology, Toranomon Hospital, Tokyo; and [7]Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan

Background. Pegylated interferon, ribavirin, and telaprevir triple therapy is a new strategy expected to eradicate the hepatitis C virus (HCV) even in patients infected with difficult-to-treat genotype 1 strains, although adverse effects, such as anemia and rash, are frequent.

Methods. We assessed efficacy and predictive factors for sustained virological response (SVR) for triple therapy in 94 Japanese patients with HCV genotype 1. We included recently identified predictive factors, such as IL28B and ITPA polymorphism, and substitutions in the HCV core and NS5A proteins.

Results. Patients treated with triple therapy achieved comparatively high SVR rates (73%), especially among treatment-naive patients (80%). Of note, however, patients who experienced relapse during prior pegylated interferon plus ribavirin combination therapy were highly likely to achieve SVR while receiving triple therapy (93%); conversely, prior nonresponders were much less likely to respond to triple therapy (32%). In addition to prior treatment response, IL28B SNP genotype and rapid viral response were significant independent predictors for SVR. Patients with the anemia-susceptible ITPA SNP rs1127354 genotype typically required ribavirin dose reduction earlier than did patients with other genotypes.

Conclusions. Analysis of predictive factors identified IL28B SNP, rapid viral response, and transient response to previous therapy as significant independent predictors of SVR after triple therapy.

Hepatitis C virus (HCV) establishes a chronic infection in 80% of infected individuals, and currently, >100 million persons are chronically infected and at increased risk of cirrhosis, hepatocellular carcinoma, and end-stage liver disease [1–3]. The current standard of care is combination treatment with pegylated interferon (PEG-IFN) and ribavirin, but this costly and poorly tolerated treatment achieves sustained virological response in only 50% of patients [4]. Options are limited in the event of treatment failure, and alternative therapies are needed.

Of the many drugs under investigation, the most promising are the direct-acting antiviral agents, which directly target essential aspects of viral replication, including internal ribosome entry site inhibitors, protease and polymerase inhibitors, and assembly inhibitors [5]. Several protease inhibitors, including telaprevir and boceprevir, are in phase III clinical trials and will likely become the first direct-acting antiviral agents approved for clinical use [6].

Received 25 November 2010; accepted 11 February 2011.

Potential conflicts of interest: none reported.

Correspondence: Kazuaki Chayama, MD, PhD, Dept of Medical and Molecular Science, Div of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan (chayama@hiroshima-u.ac.jp).

The Journal of Infectious Diseases 2011;204:84–93

0022-1899 (print)/1537-6613 (online)/2011/2041-0013$14.00
DOI: 10.1093/infdis/jir210

The HCV genome is initially translated as a large polyprotein and must be processed to produce functional viral proteins. Host proteases cleave the N-terminal structural proteins, but the viral NS3-4A serine protease is essential for cleaving the nonstructural proteins. NS3-4A also interferes with the immune response by degrading immune-signaling molecules [7]. Consequently, targeting this protease using the peptidomimetic inhibitor telaprevir both interferes with viral replication and may help rescue immune signaling, leading to a rapid decrease in HCV RNA level [8, 9]. In most patients, however, viral decline after telaprevir monotherapy is short-lived, followed by viral breakthrough because of strong selection for escape mutants within several weeks. Combination therapy with IFN alone yields unsatisfactory results, and ribavirin appears to be required to avoid relapse [10]. Because telaprevir triple therapy is an extension of the current standard of care instead of an IFN-free alternative, it does not address problems associated with the cost or adverse effects of combination therapy and may limit options for retreatment; however, it is particularly promising for patients who showed at least a transient response after prior combination therapy [11]. Nonetheless, telaprevir monotherapy may provide an alternative treatment for patients unable to tolerate IFN and/or ribavirin—at least in patients with low viral loads [12]. Additional research is needed to identify factors predicting outcome of treatment and incidence of adverse effects in different populations.

A number of host factors are known to affect outcome of PEG-IFN plus ribavirin combination therapy, including age, fibrosis, obesity, hepatic steatosis, [13] low-density lipoprotein cholesterol, γ-gamma-glutamyl transpeptidase (GTP) [14], and insulin resistance [15]. A number of recent studies have also shown that common genetic variation in the IL28B locus on chromosome 19 is strongly associated with spontaneous clearance and outcome after combination therapy [16–19]. Viral factors have also been shown to predict response to combination therapy, including HCV genotype [20], baseline viral titer [13, 20], amino acid substitutions at positions 70 and 91 of the HCV core protein, and the NS5A IFN Sensitivity Determining Region (ISDR) [21, 22]. Because telaprevir directly targets the virus and often results in selection for escape mutants, it is likely that additional predictive factors affecting response to treatment will be uncovered.

Combination therapy is poorly tolerated among some patients, and ribavirin-induced anemia is a serious adverse effect of the therapy that may result in dose reduction or discontinuation. Recent studies have shown an association between genetic variation in the ITPA locus and change in hemoglobin levels during treatment [23–25]. Although it does not appear to affect outcome of therapy [23, 24] (but see [25]), patients with an anemia-susceptible genotype may require greater reductions in ribavirin dose, which is associated with poorer response to therapy [26]. Telaprevir also moderately affects hemoglobin levels, but rash is the most common side effect of telaprevir therapy [10].

In the current study, we examined 94 patients with genotype 1 who received triple therapy to identify predictors for response to treatment and to assess effects of triple therapy on hemoglobin levels.

METHODS

Patients

Ninety-four Japanese patients who participated in a phase 3 clinical trial of the triple therapy in 2010 at Hiroshima University Hospital, Sapporo Kosei Hospital, and Toranomon Hospital (16, 17, and 61 patients, respectively) were investigated. Inclusion criteria for the study included remaining positive for genotype 1 HCV RNA for >6 months; having an HCV RNA level ≥5.0 log IU/mL, as determined by the COBAS TaqMan HCV test (Roche Diagnostics KK); and being aged 20–65 years, with a body weight >40 kg and <120 kg at the time of entry into the study. Exclusion criteria included cirrhosis; results positive for hepatitis B surface antigen or antibody against HIV; previous or current hepatocellular carcinoma; possible overlapping liver diseases, such as autoimmune hepatitis, hemochromatosis, Wilson disease, alcoholic liver disease, or renal disease; or creatinine clearance ≤50 mL/min at baseline, hemoglobin level <12 g/dL, neutrophil count <1500 neutrophils/mm^3, or platelet count <100,000 platelets/mm^3 at baseline. Patient profiles are shown in Tables 1 and 2.

All patients were treated with PEG-IFN-α-2b, ribavirin, and telaprevir triple therapy. Telaprevir (750 mg; MP-424; Mitsubishi Tanabe Pharma) was administered every 8 h after meals. PEG-IFN-α-2b (Schering Plough) was injected subcutaneously at a median dose of 1.5 μg/kg per week. Ribavirin (Schering Plough) dose was adjusted by body weight (600 mg for ≤60 kg; 800 mg for >60 to ≤80 kg; and 1000 mg for >80 kg), based on guidelines by the Ministry of Health, Labor and Welfare of Japan [27], and the drug was administered orally after breakfast and dinner. Triple therapy with telaprevir was given for 12 weeks, followed by an additional 12 weeks of PEG-IFN-α-2b and ribavirin combination therapy. Triple therapy was withdrawn if hemoglobin levels were <8.5 g/dL. Ribavirin dose was reduced by 200 mg/day in patients who were receiving 600 or 800 mg/day (or by 400 mg in those receiving 1000 mg/day) when hemoglobin levels decreased to <12 g/dL and by an additional 200 mg if levels decreased to <10 g/dL. In addition, ribavirin dose was also reduced by 200 mg in patients with a hemoglobin level <13 g/dL at baseline and in those in whom the level decreased by 1 g/dL to <13 g/dL within 1 week. PEG-IFN dose was decreased to one-half when leukocyte count decreased to <1500 leukocytes/mm^3, neutrophil count decreased to <750 neutrophils/mm^3, or platelet count decreased to <80 × 10^3 platelets/mm^3; PEG-IFN was withdrawn if these factors decreased to <1000 leukocytes/mm^3, 500 neutrophils/mm^3, or 50 × 10^3 platelets/mm^3, respectively. Triple therapy was suspended temporarily when

Table 1. Patient Characteristics

	Total (n = 94)	SVR (n = 69)	Non-SVR (n = 25)
Response to previous therapy (naive/relapser/NR)	25/44/25	20/41/8	5/3/17
Age	57 (23–65)	57 (23–65)	56 (40–65)
Sex (M/F)	52/42	42/27	10/15
Height (cm)	163.6 (141.8–189.2)	164.7 (141.8–189.2)	157.7 (148.5–181.5)
Weight (kg)	61 (41–92.5)	61.7 (41–92.5)	58.8 (44.9–80.3)
rs8099917 (TT/TG/GG)	50/41/3	47/21/1	3/20/2
rs1127354 (CC/CA/AA)	75/18/1	55/13/1	20/5/0
Viral genotype (1b/others)	93/1	69/0	24/1
Core 70 (W/M/ND)	50/43/1	43/26/0	7/17/1
Core 91 (W/M/ND)	48/45/1	39/30/0	9/15/1
ISDR (0–1/≥2/ND)	82/8/4	61/5/3	3/21/1
WBC (/mm^3)	4800 (2800–8100)	4900 (2800–8100)	4660 (3000–7900)
Plt ($\times 10^4$/mm^3)	17.7 (9.1–33.8)	18 (9.9–33.8)	16 (9.1–23.9)
Hb (g/dL)	14.3 (12.3–16.6)	14.5 (12.5–16.5)	14.1 (12.3–16.6)
ALT (IU/L)	39 (12–302)	38 (12–302)	46 (17–135)
γGTP (IU/L)	36 (11–233)	33 (11–233)	53 (19–226)
Virus titer (log IU/mL)	6.7 (5.1–7.7)	6.8 (5.1–7.7)	6.7 (5.4–7.6)
Days to first ribavirin reduction	17 (2–168)	18 (2–168)	14 (7–73)
Duration of telaprevir administration (days)	85 (29–85)	85 (29–85)	84 (35–85)
Duration of peg-interferon injection (days)	162 (22–165)	162 (22–165)	162 (30–165)
Duration of ribavirin administration (days)	169 (29–169)	169 (29–169)	168 (36–169)
Effect of therapy (SVR/BT/TR/NR)	69/4/19/2	–	–

NOTE. All patients were infected with genotype 1. Counts are listed for categorical values and the median and range are reported for continuous variables. ND, not determined, data unavailable.

hemoglobin levels decreased to <8.5 g/dL. Treatment was resumed with PEG-IFN and 200 mg ribavirin if hemoglobin levels increased to ≥8.5 g/dL within 2 weeks after withdrawal. Reduction of telaprevir dose was not permitted. It was discontinued if severe adverse effects appeared, and therapy was continued with PEG-IFN and ribavirin alone. Erythropoietin was not used to elevate hemoglobin levels.

Virologic response was analyzed on an intent-to-treat basis. The successful end point of treatment was sustained virological response (SVR) for patients who showed undetectable HCV RNA for 24 weeks after cessation of treatment. In transient responders (or persons who experienced relapse), HCV RNA levels became undetectable by the end of treatment but became positive again during the follow-up period. In patients with viral breakthrough, HCV RNA became undetectable during the treatment period but then became positive again before the end of the treatment period. The remaining patients whose HCV RNA never became undetectable were nonresponders. We also defined rapid virological response (RVR) as undetectable HCV RNA at week 4 of treatment and early virological response as a >2 $\log_{10}$ decrease in HCV RNA levels by week 12 of treatment. All participants gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA Levels

HCV RNA levels were measured using the TaqMan reverse-transcription polymerase chain reaction (PCR) test. The measurement range of this assay was 1.2–7.8 log IU/mL. Samples that exceeded the measurement range were diluted with phosphate-buffered saline and reanalyzed.

ISDR and Core Amino Acid Substitutions

Amino acid substitutions in the HCV core and ISDR regions were determined using direct sequencing of PCR products after extraction and reverse transcription of HCV RNA with use of serum samples kept frozen at −80°C. Core amino acid substitutions at positions 70 and 91 (core70 and core91, respectively) were determined according to Akuta et al [14, 28], and the number of ISDR substitutions was determined using the methods of Enomoto et al [21, 29, 30].

Single-Nucleotide Polymorphism (SNP) Genotyping

We genotyped each patient for 2 SNPs: rs8099917, an IL28B SNP previously reported to be associated with therapy outcome, and rs1127354 [31], an ITPA SNP reported to be associated with ribavirin-induced anemia [23]. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or with the Invader or TaqMan assay, as described elsewhere [32, 33].

Table 2. Patient Characteristics Grouped by Treatment History

	Total (n = 94)	Naive (n = 25)	Relapser (n = 44)	NR (n = 25)
Age	56.5 (23–65)	54 (23–64)	57.5 (44–65)	57 (40–65)
Sex (M/F)	52/42	13/12	27/17	12/13
Height (cm)	163.5 (142–189)	163 (147–189)	167.5 (142–177)	160 (149–174)
Weight (kg)	61 (41–93)	57 (42–80)	63.5 (41–93)	59 (45–77)
rs8099917 (TT/GT/GG)	50/41/3	15/9/1	33/11/0	2/21/2
rs1127354 (CC/CA/AA)	75/18/1	18/6/1	34/10/0	23/2/0
Viral genotype (1b/others)	93/1	25/0	44/0	24/1
Core 70 (W/M/ND)	50/43/1	13/12/0	28/16/0	9/15/1
Core 91 (W/M/ND)	48/45/1	14/11/0	23/21/0	11/13/1
ISDR (0–1/≥2/ND)	82/8/4	25/0/0	38/4/2	19/4/2
WBC (/mm^3)	4800 (2800–8100)	5390 (3000–7500)	4750 (2800–8100)	4700 (3040–8000)
Plt ($\times 10^4$/mm^3)	18 (9–34)	20 (15–30)	16.5 (10–34)	16 (9–24)
Hb (g/dL)	14.3 (12.3–17)	14.1 (12.5–16.1)	14.45 (12.3–17)	14.4 (12.3–16.6)
ALT (IU/L)	38.5 (12–302)	35 (12–113)	39.5 (16–302)	45 (17–135)
γGTP (IU/L)	36 (11–233)	31 (11–141)	34 (14–233)	49 (21–226)
Virus titer (log IU/mL)	6.7 (5.1–7.7)	6.7 (5.1–7.4)	6.7 (5.4–7.6)	6.7 (5.8–7.7)
Days to first ribavirin reduction	18 (3–168)	18 (3–52)	18 (3–168)	15 (8–52)
Duration of telaprevir administration (days)	85 (29–85)	85 (29–85)	85 (32–85)	85 (35–85)
Duration of peg-interferon injection (days)	162 (22–165)	163 (22–165)	162.5 (30–165)	162 (30–165)
Duration of ribavirin administration (days)	169 (29–169)	168 (29–169)	169 (32–169)	169 (36–169)
Effect of therapy (SVR/BT/TR/NR)	69/4/19/2	20/0/5/0	41/1/2/0	8/3/12/2

NOTE. Counts are listed for categorical values and the median and range are reported for continuous variables.

Statistical Analysis

Statistical analysis was performed using PASW Statistics, version 18 (SPSS) and R, version 2.11. Categorical data were analyzed using χ^2 and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann-Whitney U test. To identify independent predictive factors, variables that were significant at the .05 level in univariate tests were considered as candidate factors for multiple logistic regression analysis. The model was reduced using AIC-based forward and/or backward stepwise selection with bootstrap validation. Odds ratios (ORs) were corrected for over-optimism with use of penalized maximum likelihood.

RESULTS

Effect of the Triple Therapy by Previous Response to PEG-IFN Plus Ribavirin Therapy

Patient profiles are shown in Tables 1 and 2. After triple therapy, 69 (73%) of 94 patients achieved SVR. Of the 25 treatment-naive patients, 20 (80%) eradicated the virus, and the remaining 5 achieved transient response. Similarly, 49 (71%) of the 69 patients who had received prior treatment achieved SVR with triple therapy. Of note, however, 41 (93%) of 44 patients who had responded transiently to previous treatment were able to eradicate the virus with use of triple therapy. Conversely, only 8 (32%) of 25 patients who had failed to respond to prior treatment were able to achieve SVR with use of triple therapy, and 2 of these patients also failed to respond to triple therapy. None of the 4 patients in whom viral breakthrough occurred were treatment naive, and 3 of the 4 were nonresponders to prior treatment.

IL28B SNP Genotypes

The genotype of IL28B SNP rs8099917 was determined for each patient. The frequency of the rs8099917 risk allele (G) was 0.25 among all patients, 0.17 among patients who achieved SVR, 0.38 among patients with viral breakthrough, and 0.5 among both transient responders and nonresponders. Patients with the rs8099917 TT genotype were significantly more likely to achieve SVR (94% vs 50%; P = 4.6E-6; Figure 1) and had significantly higher baseline viral loads (6.9 vs 6.45 log IU/mL; P = .0056; Figure 2D), compared with patients with GT or GG genotypes.

Loss of Hemoglobin During and After Triple Therapy

The triple therapy resulted in hemoglobin loss in all patients, but the pattern differed by ITPA SNP rs1127354 genotype (Figure 3). The frequency of the rs1127354 minor allele (A) was 0.11 among all patients, 0.11 among patients who achieved SVR, .13 among transient responders, and 0 in both patients with viral breakthrough and nonresponders. There was no effect of rs1127354 genotype on SVR (73% for both CC and non-CC genotypes), but ribavirin dosage reduction was required significantly earlier in patients with genotype CC than in those with non-CC genotypes (18 days vs 29 days, respectively; P = 3.2E-5; Figure 4). Although hemoglobin loss